-- This is a divisional of application Serial No. 08/925,676 filed September 9, 1997, now U.S. Patent No. 6,054,270, which is a divisional of application Serial No. 08/230,012, filed April 19, 1994, now U.S. Patent No. 5,700,637, which is a continuation of abandoned application Serial No. 07/695,682, filed May 3, 1991, which is a continuation-in-part of abandoned application Serial No. 07/573,317, filed September 28, 1990, which is a 371 of PCT/GB89/00460, filed May 2, 1989. --

Page 7, line 3, change "the table" to -- The table --.

Page 9, line 30, change "occurrances" to -- occurrences --.

Page 13, line 21, change "positons" to -- positions --.

## IN THE ABSTRACT

Please add the attached Abstract consisting of a single sheet.

## REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and following remarks.

A substitute specification was required. Submitted herewith is a substitute specification consisting of pages 1-21 which corresponds identically to the specification as originally filed consisting of pages 1-30. The substitute specification contains no new matter.

The foregoing amendments to the substitute specification identify the parent applications and issued patents. In addition, minor typographical errors appearing in the original specification and duplicated in the substitute specification have been corrected which are self-explanatory.

The Action required a new Oath or Declaration, on the basis that the copy of the Declaration filed was copied so close to the top that whole punching to assemble the application file punched out some of the top wording.

Rather then providing a new Declaration or Oath, Applicants enclose a new copy of the Declaration as originally filed which is believed to overcome the problem indicated in the Action.

The Action stated a rejection of claim 1 on the basis that statutory double patenting as well as a rejection of claim 1 under 35 USC 102 as being anticipated by Mundy and Saiki et al. and Brigati et al.

These grounds of rejection are deemed to be overcome by the presentation of the new claims. As indicated in the Preliminary Amendment filed concurrently with this divisional application, the present application is intended to be directed to certain claims which were cancelled without prejudice in the parent application Serial No. 08/925,676. Applicants had intended to file a preliminary paper prior to issuance of the first Action, formally presenting such claims prior to issuance. Unfortunately, the claims were not finalized prior to issuance of the first Action.

As noted above, claim 1 has been cancelled without prejudice and replaced with new claims 17-99.

For the Examiner's information, the above new claims correspond to claims filed in Serial No. 08/925,676 as follows:

New claim 17 corresponds to claim 17.
New claim 18 corresponds to claim 110.
New claim 19 corresponds to claim 26.
New claim 20 corresponds to claim 18.
New claim 21 corresponds to claim 19.
New claim 22 corresponds to claim 20.
New claim 23 corresponds to claim 21.
New claim 24 corresponds to claim 22.

New claim 25 corresponds to claim 23.

New claim 26 corresponds to claim 24.

New claim 27 corresponds to claim 25.

New claim 28 corresponds to claim 112.

New claim 29 corresponds to claim 113.

New claim 30 corresponds to claim 114.

New claim 31 corresponds to claim 100.

New claim 32 corresponds to claim 101.

New claim 33 corresponds to claim 102.

New claim 34 corresponds to claim 84.

New claim 35 corresponds to claim 85.

New claim 36 corresponds to claim 27.

New claim 37 corresponds to claim 104.

New claim 38 corresponds to claim 106.

New claim 39 corresponds to claim 107.

New claim 40 corresponds to, and is a combination of, claims 36 and 37.

New claim 41 corresponds to, and is a combination of, claims 36 and 38.

New claim 42 corresponds to claim 111.

New claim 43 corresponds to claim 108.

New claim 44 corresponds to claim 39.

New claim 45 corresponds to claim 40.

New claim 46 corresponds to claim 41.

New claim 47 corresponds to claim 42.

New claim 48 corresponds to claim 43.

New claim 49 corresponds to claim 97.

New claim 50 corresponds to claim 98.

New claim 51 corresponds to claim 99.

New claim 52 corresponds to claim 44.

New claim 53 corresponds to claim 45.

New claim 54 corresponds to claim 46.

New claim 55 corresponds to claim 109.

New claim 56 corresponds to claim 92.

New claim 57 corresponds to, and is a combination of claims 86 and 87.

New claim 58 corresponds to, and is a combination of claims 47 and 53.

New claim 59 corresponds to claim 82

New claim 60 corresponds to claim 83.

New claim 61 corresponds to claim 96.

New claim 62 corresponds to claim 103.

New claims 63 and 64 correspond to, and are a combination of claims 55 and 90.

New claim 65 corresponds to, and is a combination of claims 55 and 56.

New claim 66 corresponds to claim 57.

New claim 67 corresponds to claim 58.

New claim 68 corresponds to claim 60.

New claim 69 corresponds to claim 61.

New claim 70 corresponds to claim 93.

New claim 71 corresponds to claim 62.

New claim 72 corresponds to claim 63.

New claim 73 corresponds to claim 64.

New claim 74 corresponds to claim 64.

New claim 75 corresponds to claim 65.

New claim 76 corresponds to claim 66.

New claim 77 corresponds to claim 94.

New claim 78 corresponds to claim 67.

New claim 79 corresponds to claim 68.

New claim 80 corresponds to claim 73.

New claim 81 corresponds to claim 74.

New claim 82 corresponds to claim 79.

New claim 83 corresponds to claim 80.

New claim 84 corresponds to claim 81.

New claim 85 corresponds to claim 91.

New claim 86 corresponds to claim 89.

New claim 87 corresponds to claim 87.

New claim 88 corresponds to claim 59.

New claim 89 corresponds to claim 69.

New claim 90 corresponds to claim 70.

New claim 91 corresponds to claim 75.

New claim 92 corresponds to claim 76.

New claim 93 corresponds to claim 77.

New claim 94 corresponds to claim 78.

New claim 95 corresponds to, and is a combination of claims 71 and 88.

New claims 96-99 are new kit claims.

Support in Original Specification for Proposed Claims

Of the claims which were filed at the USPTO on 26 November 1997 in Serial No. 08/925,676, claims 18-35, 37-46, 53, 56-70, 73,74, 79-85, 87-94, 96-104 and 106-114 were subsequently rejected. In addition, claims 17 and 75 (together with 19, 24, 25 and 76-78) were rejected as being unpatentable over a single prior art reference, Singer et al. The proposed new claims are based upon these claims. Please see the specification **as originally filed** as outlined in the following paragraphs for the requisite support.

New claim 17 is supported in the specification, particularly on pages 1-3, page 10 at lines 31-33:

"These 46,377 positive cells represented known sequences, determined from their position in the matrix"

and page 11 at lines 10-14 and 25.:

"The method described here envisages that the matrix will be produced by synthesising oligonucleotides in the cells of an array by laying down the precursors for the four bases in a predetermined pattern, an example of which is described above."

New claim 18 is supported in the specification as described above and furthermore at page 8. The lower limit of the claim i.e. 72 different known locations is supported at page 19, example 3, which states:

"For a further study of the effects of mismatches or shorter sequences on hybridisation behaviour, we constructed two arrays; one (a) of 24 oligonucleotides and the other (b) of 72 oligonucleotides."

The upper limit is supported by the data in the table on page 8, i.e. $1.1 \times 10^{12}$ in the $4^8$ column.

New claim 19 is supported as described above and furthermore at page 5, lines 12-18.
All of the elements of claim 19 are supported by the specification

*"An array of oligonucleotides for analysing mutations of a gene having a known nucleotide sequence... "*

The claim is primarily supported by the paragraph bridging pages 4 and 5 which describes analysis of a known sequence, with reference to base changes (mutations) in human genes.

*"...comprising a support having an impermeable surface to which are attached at different known locations overlapping (oligonucleotides)... "*

Supported by the bridging paragraph on page 4 which reads:

"Any known sequence can be represented completely **(on an array)** as a set of *overlapping oligonucleotides*. The size of the set is Ns + 1 = N, where N is the length of the sequence and s is the length of an oligomer. A gene of 1 kb for example, may be divided into an overlapping set of around one thousand oligonucleotides of any chosen length. An array constructed with each of these oligonucleotides in a separate cell can be used as a multiple hybridisation probe to examine the homologous sequence in any context" (*emphasis added*) **(added)**

*"... or partly overlapping or non-overlapping oligonucleotides... "*

Page 5, line 12-18 reads:

"For a less complete analysis it would be possible to reduce the size of the array e.g. by a factor of up to 5 by representing the sequence in a partly or non-overlapping set. The advantage of using a completely overlapping set is that it provides a more precise location of any sequence difference, as the mismatch will scan in s consecutive oligonucleotides.

*"... which are complementary to a segment of the known nucleotide sequence of the gene... "*

This element of the claim is inherent in the disclosed method of the invention i.e. the segment of the known nucleotide sequence of the gene is a hybridising probe which is used to explore the array (page 2, line 26-31).

New claim 20 is supported at page 11, particularly lines 19-29 and page 14, lines 20-22.

This passage teaches that the pixel size or resolution of the predetermined pattern of synthesised oligonucleotides in an array would have an upper limit of 100 microns and a lower limit of 10 microns for example where a laser typesetter is used.

New claim 21 is supported in the paragraph bridging pages 1-2 of the specification.

This paragraph states that the invention provides an array of the whole or chosen part of a complete set of oligonucleotides of chosen lengths. It therefore gives full support to the claim.

New claim 22 is supported for example on the paragraph bridging pages 10-11.

This part of the specification describes the computer simulation of the use of an array of 10 mers derived from a predetermined sequence i.e. the genome of lambda phage. This therefore supports claim 22. The claim is further supported at p 28, line 10, which states that for some applications the array should be a predetermined limited set and that for other applications the array should comprise every sequence of a predetermined length. This passage also gives support to claim 5.

New claim 23 is supported in the paragraph bridging pages 11-12, particularly page 11 at line 18.

This passage clearly states that the matrix - the array can be a square matrix (page 8, line 2) - can be produced by laying down oligonucleotide precursors by means of automatic equipment e.g. computer controlled printing devices. Further support is found at page 22 where in example 5, a pen-plotter, controlled by a microcomputer is used to deliver oligonucleotide synthesis chemicals to an array of twelve spots containing oligonucleotide sequences. In addition it is stated on page 26, paragraph 2, that multiple sequences can be synthesised on the sample in small spots, at high density, by a simple manual procedure, or automatically using a computer-controlled device.

New claim 24 is supported by the sentence bridging pages 11-12, which reads:

"However, a low cost ink-jet printer can print at speeds of about 10,000 spots per second. With this sort of speed, $10^8$ spots (*of oligonucleotides*) could be printed in about three hours" (*added*)

Further support is given at page 28 which states at line 4:

"We have shown that oligonucleotides can be synthesised in small patches in precisely determined positions by one of two methods: by delivering the precursors through the pen of a penplotter, or by masking areas with silicone rubber. It is obvious how a pen plotter could be adapted to synthesise large arrays with a different sequence in each position."

Furthermore, example 5 on page 22, explicitly describes the use of a penplotter to apply oligonucleotide synthesis chemicals to different known locations.

New claim 25 is supported by original claim 14 in USSN 08/925,676, which reads:

"A method as claimed in any one of claims 8 to 13, wherein the chosen length is from 8 to 20 nucleotides"

Support for both of these claims is derived from the table on page 8 which shows oligonucleotide lengths (variable's') between 8 and 20 nucleotides.

New claim 26 is supported at page 11, line 19-33, which reads:

"The smaller the pixel size of the array the better, as complex genomes need very large numbers of cells. However, there are limits to how small these can be made. 100 microns would be a fairly comfortable upper limit, but could probably not be achieved on paper for reasons of texture and diffusion. On a smooth impermeable surface, *such as glass*, it may be possible to achieve a resolution of around 10 microns, for example by using a laser typesetter to pre-form a solvent repellent grid, and building the oligonucleotides in the exposed regions. One attractive possibility, which allows adaptation of present techniques of oligonucleotide synthesis, is to sinter microprobes *glass* in microscopic patches onto the surface of a *glass* plate." (*emphasis added*)

Further support is derived from example 5.3 on page 12, which reads:

"In an experiment performed by us, CPG (*controlled pore size glass*) was used as the support in an Applied Bio-sytems (sic) oligonucleotide synthesiser to synthesise a 13-mer complementary to the left hand cos site of phage lambda. The coupling steps were all close to theoretical yield. The first base was stably attached to the support medium through all the synthesis and deprotection steps by a covalent link". (*added*)

New claim 27 is supported at page 16, line 26, which reads:

"Commercially available microscope slides (BDH Super Premium 76 x 26 x 1 mm) were used as supports."

This statement refers to the use of slides as a support in examples 1, 2, 3 and 6, where such use is described explicitly.

New claim 28 is supported on pages 6-8, particularly page 7 at lines 24-26 which states:

"If 4s is an order of magnitude greater than N, there should only be a few, (oligonucleotides) 3s/10, related to any (other) oligonucleotide by one base change."

Thus it is taught that a desired feature of the invention is to have the value of s (number of nucleotides in the oligonucleotides of the array) such that $4^s > 10N$ where N is the length of the polynucleotide to be analysed by the array, as this reduces the probability of there being oligonucleotides related to others by only one base change.

New claim 29 is supported at page 11, lines 29-35.

"One attractive possibility, which allows adaptation of present techniques of oligonucleotide synthesis, is to sinter microporous glass in microscopic patches onto the surface of a glass plate"

It would be facile for one skilled in the art to adapt a glass plate for use in the support of oligonucleotides by sintering microporous glass in discreet patches on its surface.

The amount of glass that would be desirable is also taught on page 13:

"The yield of oligonucleotides synthesised on microporous glass is about 30 $\mu$mol/g. A patch of this material 1 micron thick by 10 microns square would hold , 3 x $10^{-12}$ µmol, equivalent to about 2 g of human DNA."

New claim 30 is supported at page 15, lines 15-17, which states:

"...the amount of oligonucleotide laid down on the surface of the support can be varied depending on its nucleotide composition..."

New claim 31 is supported at page 22, line 35 to page 23, line 3, which states:

"Filling the pen successively with G, T and A phosphoramidite solutions an array of twelve spots was laid down in three groups of four, with three different oligonucleotide sequences"

The art skilled would understand that the groups of nucleotides resulting from the above procedure differ by single nucleotide residues, thus this passage supports the claim.

New claim 32 is supported at page 27, lines 9-17, which states:

"In the same way as mutations which lead to disease can be detected, the method could be used to detect point mutations in any stretch of DNA. Sequences are now available for a number of regions containing the base differences which lead to restriction fragment length polymorphisms (RFLPs). An array of oligo-nucleotides representing such polymorphisms could be made from pairs of oligonucleotides representing the two allelic restriction sites.

Amplification of the sequence containing the RFLP, followed by hybridisation to the plate, would show which alleles were present in the test genome"

As outlined in the above passage, an array of pairs of oligonucleotides representing the two allelic restriction sites results in an array of allelic polymorphisms as found in recited in claim 32. Thus the claim is fully supported.

New claim 33 is supported at page 27, lines 22-24, which continues from the above passage to state:

"The number of oligonucleotides that could be analysed in a single analysis could be quite large. Fifty pairs made from selected alleles would be enough to give a fingerprint unique to an individual."

New claim 34 is supported at page 13, lines 5-9 which reads:

"Where sequence variations are known, an advantage of using this technique is that many different mutations can be probed simultaneously by laying down stripes corresponding to each allelic variant"

Thus the recited element of the claim i.e. stripes corresponding to allelic variants, for simultaneous probing, is given full support.

New claim 35 is supported at page 13, lines 9-10 and page 29, line 1, which reads:

"With a density of one oligonucleotide per mm, and one "individual" per 5 mm, it should be possible to analyse 2000 loci on a plate 100 mm square. Such a high density of information, where the oligonucleotides do identify specific alleles, is not available by other techniques."

New claim 36 is supported at page 4, lines 26 and 30. The application of the array of the invention to the problems of scanning for multiple mutations and confirming mutations suggested by linkage analysis is disclosed for the genes and disease loci mentioned in the above passage i.e. the DMD and HPRT genes and the Huntingdon's disease and Cystic Fibrosis loci. This claim is also supported by example 4, on page 21, and example 6 on page 23, which both successfully test the application of the invention to diagnosis of inherited diseases.

New claim 37 is supported at page 29, lines 3-26, which states:

"There are useful applications for arrays in which part of the sequence is predetermined and part made up of all possible sequences. For example:

**Characterising mRNA populations.**

Most mRNAs in higher eukaryotes have the sequence AAUAAA close to the 3' end. The array used to analyse mRNAs would have this sequence all over the plate. To analyse a mRNA population it would be hybridised to an array composed of all sequences of the type NmAATAAANn. For m + n = 8, which should be enough to give a unique oligonucleotide address to most of the several thousand mRNAs that is estimated to be present in a source such as a mammalian cell, the array would be 256 elements square. The 256 x 256 elements would be laid on the AATAAA using the masking method described above. With stripes of around 1 mm, the array would be ca. 256mm square.

This analysis would measure the complexity of the mRNA population and could be used as a basis for comparing populations from different cell types. The advantage of this approach is that the differences in the hybridisation pattern would provide the sequence of oligonucleotides that could be used as probes to isolate all the mRNAs that differed in the populations."

New claim 38 is supported as discussed above for claim 19.

New claim 39 is supported throughout the examples, but particularly in example 5.3 on page 12 which reads:

"The first base was stably attached to the support medium through all the synthesis and deprotection steps by a covalent link."

New claim 40 is supported at page 12, lines 7-16, which reads:

"... there are reports of the use of oligonucleotides as hybridisation probes on solid supports to which they were attached after synthesis."

This statement tells the skilled reader that oligonucleotides attached to a solid support after synthesis can be used as hybridisation probes and hence may be used in an array of the invention. There is no material reason why pre-synthesized oligonucleotides could not be attached to a solid support to make an array and this is taught by the presence of the above phrase in the specification.

New claim 41 is supported by examples 1 and 2.

Example 1 discloses the synthesis of oligonucleotide on a derivatised slide, which is used as a support for the array. The skilled reader would understand that this discloses that "*oligonucleotides are synthesized in situ on the surface of the support*".

The phrase *in situ* would be understood to mean, on the support to be used as an array and in the different known locations of that array, this is because in examples 1, 2, 3, 5 the in situ synthesis of oligonucleotides on a solid support in different known locations is explicitly disclosed.

New claim 42 is supported as discussed above for claim 18.

New claim 43 is supported as discussed above for claim 17.

New claims 44-48 are supported as discussed for claims 20-24 above.

New claim 49 is supported at page 22, lines 23-27, which states:

"The pen of the plotter had been replaced by a component, fabricated from Nylon, which had the same shape and dimensions as the pen, but which carried a polytetrafluoroethylene (PTFE) tube"

New claim 50 is supported at page 22, line 33-35, which states

"The pen, carrying the delivery tube from the syringe, was moved into position above the slide, the pen was lowered and the pump activated to lay down coupling solution."

New claim 51 is supported at page 22, line 35 to page 23, line 3, which states:

"Filling the pen successively with G, T and A phosphoramidite solutions an array of twelve spots was laid down in three groups of four, with three different oligonucleotide sequences."

The skilled reader would understand that the groups of nucleotides resulting from the above procedure differ by single nucleotide residues, thus this passage supports the claim.

New claims 52-54 is supported as discussed for claims 25-27 above.

New claim 55 is supported as discussed above for claim 39.

New claim 56 is supported as described above for claim 30.

New claim 57 is supported at page 13, lines 5-9 which reads:

"Where sequence variations are known, an advantage of using this technique is that many different mutations can be probed simultaneously by laying down stripes corresponding to each allelic variant"

and page 28, line 34 to page 29 line 1, which reads:

"The dimensions of such arrays are determined by the width of the stripes. The narrowest stripe we have laid is 1 mm, but this is clearly not the lowest limit."

New claim 58 is supported on pages 6-8, and particularly at page 11, lines 26-29, which states:

"On a smooth impermeable surface, such as glass, it may be possible to achieve a resolution of around 10 microns, for example by using a laser typesetter to preform a solvent repellent grid, and building the oligonucleo-tides in the exposed regions"

Also the use of a solvent repellent grid is shown by the examples, in particular example 3 which the solvent repellent grid with exposed regions for building oligonucleotides is formed with silicone rubber tubing.

New claim 59 is supported as discussed above for claim 58.

New claim 60 is supported as discussed above for claim 29.

New claim 61 is supported at page 19, line 30 to page 20, line 11, which states:

"These arrays were set out as shown in Table 1(a) and 1(b). The masks used to lay down these arrays were different from those used in previous experiments. Lengths of silicone rubber tubing (1 mm o.d.) were glued with silicone rubber cement to the surface of plain microscope slides, in the form of a "U". Clamping these masks against a derivatised microscope slide produced a cavity into which the coupling solution was introduced through a syringe. In this way only the part of the slide within the cavity came into contact with the phosphoramidite solution. Except in the positions of the mismatched bases, the arrays listed in Table 1 were laid down using a mask which covered most of the width of the slide. Off-setting this mask by 3mm up or down the derivatised slide in subsequent coupling reactions produced the oligonucleotides truncated at the 3' or 5' ends."

And at page 29 lines 13-34 which states:

"The masking method can be used for the latter by laying down the precursors in a mask which produces intersecting lines. There are many ways in which this can be done and we give one example for illustration:

1. The first four bases, A, C, G, T, are laid in four broad stripes on a square plate.
2. The second set is laid down in four stripes equal in width to the first, and orthogonal to them. The array is now composed of all sixteen dinucleotides.
3. The third and fourth layers are laid down in four sets of four stripes one quarter the width of the first stripes. Each set of four narrow stripes runs within one of the broader stripes. The array is now composed of all 256 tetranucleotides.
4. The process is repeated, each time laying down two layers with stripes which are one quarter the width of the previous two layers. Each layer added increases the length of the oligonucleotides by one base, and the number of different oligonucleotide sequences by a factor of four."

Thus the three elements of the claim, namely applying a mask, off setting the mask and repeating said offsetting are all supported.

New claim 62 is supported at page 28, lines 4-8, which states:

"We have shown that oligonucleotides can be synthesised in small patches in precisely determined positions by one of two methods: by delivering the precursors through the pen of a penplotter, or by masking areas with silicone rubber" (emphasis added)

New claims 63 and 64 are supported at page 14, lines 9-10 which states that the polynucleotide sequence to be analysed may be of DNA or RNA.

New claim 65 is supported for example at page 6, lines 6-15 an in particular lines 11-15 which states:

"Positions in the array which are occupied by one sequence but not by the other show differences in two sequences. This gives the sequence information needed to synthesise probes which can then be used to isolate clones of the sequence involved."

New claims 66 and 67 is supported at page 5, line 29 to page 6 line 11 which states:

"In many cases, the full sequence of the nucleic acids need not be determined; the important sequences are those which differ between two nucleic acids. To give three examples: the DNA sequences which are different between a wild type organism and one which carries a mutant can lead the way to isolation of the relevant gene; similarly, the sequence differences between a cancer cell and its normal counterpart can reveal the cause of transformation; and the RNA sequences which differ between two cell types point to the functions which distinguish them. These problems can be opened to molecular analysis by a method which identifies sequence differences. Using the approach outlined here, such differences can be revealed by hybridising the two nucleic acids, for example the genomic DNA of the two genotypes, or the mRNA populations of two cell types to an array of oligonucleotides which represent all possible sequences"

New claim 68 is supported as discussed below with respect to claim 88 and furthermore at page 9, lines 14-21.

The passage at page 9, lines 14-21, provides basis for the claim element of "5% labelled cells". In this passage it is stated that the optimum condition for the first step in a sequencing strategy is to fill about 5% of cells in the matrix because at this size, a high proportion of the positive signals would represent single occurrences of each oligomer. A sequencing strategy compatible with this optimum condition is further described at page 9 line 22 to page 10, line 18. This passage provides support for the second element of the claim, that is the extension of the oligonucleotides that gave a positive signal in the first step by one base in each direction. The repetition of this procedure is also disclosed "until the sequence was complete" (page 10, line 9).

The claim element of repeating extension of the oligomers of the array until no repeated sequences are identified is also supported at page 10, line 27 to page 11, line 8. This passage reads:

> "After hybridisation,, of the lambda 10-mers in the computer, 46,377 cells were positive, 1957 had double occurrences, 75 triple occurrences, and three quadruple occurrences. These 46,377 positive cells represented known sequences, determined from their position in the matrix. Each was extended by four x one base at the 3' end and four x one base at the 5', end to give 16 x 46,377 = 742,032 cells. This extended set reduced the number of double occurrences to 161, *a further 16-fold extension brought the number down to 10, and one more provided a completely overlapped result.* Of course, the same end result of a fully overlapped sequence could be achieved starting with a 416 matrix, but the matrix would be 4000 times bigger than the matrix needed to represent all 10-mers, and most of the sequence represented on it would be redundant." (*emphasis added*).

New claim 69 is supported as discussed above regarding claim 68.

New claim 70 is supported at page 15, lines 17-19, which states:

> "...or the computer used to analyse the data can be programmed to compensate for variations in nucleotide composition."

New claims 71 and 72 are supported at page 21, lines 24-27.

The passage at page 21, lines 24-27, explicitly describes the PCR amplification of the polynucleotide to be analysed by the array, in this case a 110 by fragment of the β-globin gene. Example 6, page 23, also describes use of PCR to amplify the polynucleotide to be analysed.

Furthermore, page 2, lines 30-32 discloses that the probe (i.e. polynucleotide to be analysed) "may comprise labelled sequences amplified from genomic DNA by the polymerase chain reaction,".

New claims 73 and 74 are supported by the above passage, which continues:

"or a mRNA population, or a complete set of oligonucleotides from a complex sequence such as an entire genome"

thus giving support to these claims.

New claim 75 is supported at page 16, lines 3-23, particularly at line 6 which states "fluorescent probes are envisaged ...". It would be facile for one skilled in the art to label the probe to be analysed with a fluorescent marker rather than a radiolabel.

New claim 76 is supported: at page 14, line 17:

"If labelled with $^{32}P$, the radioactive yield of any individual s-mer even from total human DNA could be more than 104 dpm/mg of total DNA. For detection, only a small fraction of this is needed in a patch 10-100 microns square."

at page 18, example, particularly line 28:

"An autoradiograph showed that all the counts came from the area where the oligonucleotide had been synthesised, i.e. there was no non-specific binding to the glass or to the region that had been derivatised with the linker only."

at page 19, example 2, particularly line 20:

"Autoradiography showed that annealing occurred only to the part of the slide with the fully complementary oligonucleotide. No signal was detectable on the patch with the mismatched sequence."

and elsewhere in the examples.

New claims 77 and 78 are supported at page 16, lines 12-23, which states:

"We have considerable experience of scanning autoradiographic images with a digitising scanner. Our present design is capable of resolution down to 25 microns, which could readily be extended down to less than present application, depending on the quality of the hybridisation reaction, and how good it is at distinguishing absence of a sequence from the presence of one or more. Devices for measuring astronomical plates have an accuracy around 1 μ(m). Scan speeds are such that a matrix of several million cells can be scanned in a few minutes. Software for the analysis of the data is straightforward, though the large data sets need a fast computer."

New claim 79 is supported as discussed for new claim 21, and also by the paragraph bridging pages 1-2 of the specification.

This paragraph states that the invention provides an array of the whole or chosen part of a complete set of oligonucleotides of chosen lengths. It therefore gives full support to the claim.

New claims 80 and 81 are supported by the following passages:

Page 2, line 21 states:

"The chosen conditions of hybridisation and the length of the oligonucleotides must at all events be sufficient for the available equipment to be able to discriminate between exactly matched and mismatched oligonucleotides."

and Page 14, line 29 states:

"Hybridisation conditions can be chosen to be those known to be suitable in standard procedures used to hybridise to filters, but establishing optimum conditions is important. In particular, temperature needs to be controlled closely, preferably to better than ±0.5°C. Particularly when the chosen length of the oligonucleotide is small, the analysis needs to

be able to distinguish between slight differences of rate and/or extent of hybridisation. The equipment may need to be programmed for differences in base composition between different oligonucleotides. In constructing the array, it may be preferable to partition this into sub-matrices with similar base compositions. This may make it easier to define the Tm which may differ slightly according to the base composition."

New claims 82 and 83 are supported on page 15, lines 19-26 which states:

"A preferred method, which can be used either instead of or in addition to those already mentioned, is to use a chaotropic hybridisation solvent, for example a quarternary or tertiary amine as mentioned above. Tetramethylammoniumchloride (TMACI) has proved particularly suitable, at concentrations in the range 2 M to 5.5 M. At TMACI concentrations around 3.5 M to 4 M, the Tm dependence on nucleotide composition is greatly reduced."

New claim 84 is supported as discussed for claim 28.
New claim 85 is supported at page 4 lines 11-14.

"There are ways of improving the discriminating power, for example by carrying out hybridisation close to the Tm of the duplex to reduce the rate of formation of mismatched duplexes..."

and at page 14, lines 22-34, which states:

"Hybridisation conditions can be chosen to be those known to be suitable in standard procedures used to hybridise to filters, but establishing optimum conditions is important. In particular, temperature needs to be controlled closely, preferably to better than ±0.5°C.

Particularly when the chosen length of the oligonucleotide is small, the analysis needs to be able to distinguish between slight differences of rate and/or extent of hybridisation"

New claim 86 is supported at page 14, lines 1-8.

"The hybridisation reaction could therefore be carried out with a very large excess of the bound oligonucleotides over that in the probe. So it should be possible to design a system capable of distinguishing between hybridisation involving single and multiple occurrences of the probe sequence, as yield will be proportional to concentration at all stages in the reaction."

New claim 87 is supported at page 14, lines 9-10 which states that the polynucleotide sequence to be analysed may be of DNA or RNA.

New claim 88 is supported on pages 4-8.

The passage on page 6 at line 17-30 is particularly supportive:

"Sequences can be reconstructed by examining the result of hybridisation to an array. Any oligonucleotide of length s from within a long sequence, overlaps with two others over a length s-1. Starting from each positive oligonucleotide, the array may be examined for the four oligonucleotides to the left and the four to the right that can overlap with a one base displacement.

If only one of these four oligonucleotides is found to be positive to the right, then the overlap and the additional base to the right determine s bases in the unknown sequence. The process is repeated in both directions, seeking unique matches with other positive oligonucleotides in the array. Each unique match adds a base to the reconstructed sequence."

Both the claim and the passage above describe reconstruction of a sequence, by use of hybridisation to an array, locating a positive hybridisation to an oligonucleotide in the array and examining the array for further positive signals in both directions to extend the sequence information by one base at each step. Thus all of the elements of claim 72 are supported by the specification.

New claim 89 is supported as discussed for claim 19.

New claim 90 is supported as discussed for claim 36.

New claim 91 is supported for example, as discussed above for claim 1, the array, and also as discussed above for claims 68 and 88.

New claim 92 is supported as discussed above for claims 74 and 76 and is supported with new claims 93 and 94 at page 14, lines 9-16:

> "The polynucleotide sequence to be analysed may be of DNA or RNA. To prepare the probe, the polynucleotide may be degraded to form fragments. Preferably it is degraded by a method which is as random as possible, to an average length around the chosen length s of the oligonucleotides on the support, and oligomers of exact length s selected by electrophoresis on a sequencing gel. The probe is then labelled. For example, oligonucleotides of length s may be end labelled."

New claim 95 is supported as discussed for claim 91 and is further supported at page 13, lines 5-9 which reads:

> "Where sequence variations are known, an advantage of using this technique is that many different mutations can be probed simultaneously by laying down stripes corresponding to each allelic variant"

and page 28, line 34 to page 29 line 1, which reads:

"The dimensions of such arrays are determined by the width of the stripes. The narrowest stripe we have laid is 1 mm, but this is clearly not the lowest limit."

New claims 96-99 are new kit claims, the components of which are described in the specification and above.

Novelty under USC 102(e) over Singer et al.

Singer et al. deals with in situ hybridisation techniques, that is the detection of nucleic acid sequences within morphologically intact cells. By the nature of the method, in which cells are left morphologically intact, such nucleic acids are found in polynucleotide form, incorporated in chromosomal material, as messenger RNA or as viral nucleic acids (DNA or RNA). One of the features of the method of the invention is to fix the cells in such a way that the cellular morphology is left intact i.e. proteins are not crosslinked. These morphologically intact cells are fixed to the support whilst the nucleic acids are free to move within them.

The location of the cells but not the nucleic acids within them, is known prior to testing with a probe. A plurality of nucleic acids is present at each known location on the glass slide i.e. within each cell. The sequences of the nucleic acids in the target cells is not determined until confirmed by hybridisation with a probes designed against predetermined sequences thought to be present in the target cells.

Turning to the rejected claims:

Claim 17 recites an array of oligonucleotides wherein the oligonucleotide at one known location is different from the oligonucleotide at another known location. This claim is novel because this feature is not found in Singer. Whilst the cells of Singer are present in different locations, the nucleic acids of Singer are present in the same locations i.e. within each cell. In any case, the actual positions of the nucleic acids is not known until after detection, in fact the precise position of the nucleic acid cannot be determined as the nucleic acids are free to move within the usual constraints of a morphologically intact cell. Furthermore, in Singer, nucleic acids are not attached to the support, rather they are contained in a fluidic state inside the cells fixed to the support.

Claim 21 recites an array as claimed in claim 17 where in the different polynucleotides constitute part or all of a complete set of oligonucleotides of a predetermined length. It is impossible for the nucleic acids contained in the intact cells of Singer to have a predetermined length as they have not been extracted from the cell to allow determination of their length. For this reason and the reasons given above, the claim is novel over Singer.

Claim 26 recites an array as claimed in claim 17 wherein the support is made of glass.

Claim 27, which recites that the support is a glass microscope slide.

Claim 91 recites a method for determining the sequence of a polynucleotide, which comprises: applying the polynucleotide to a substrate (array), wherein the probes are immobilised at different known locations on the surface of the support such that the oligonucleotide at one known location is different from the oligonucleotide at another known location.

Thus the argument discussed above regarding the difference between an oligonucleotide at one position and the next holds true here. In addition, there are several differences between the method recited in claim 91 and the method described in Singer.

Firstly, applying polynucleotide probe in Singer would not determine the sequence of a polynucleotide, but would merely indicate that it contained a region complementary and capable of hybridising with another sequence within the fixed intact cell.

It is impossible for the nucleic acids contained in the intact cells of Singer to have a predetermined sequence as they have not been extracted from the intact cells to allow determination of their sequence. For this reason and the reasons given above, the claim is novel over Singer.

In the method disclosed by Singer non-immobilised probes of predetermined sequence are used to determine the presence or absence of hybridising nucleic acids within the chromosomal DNA and mRNA of intact cells fixed to a solid support. However, claim 91 recites that oligonucleotide probes having different predetermined sequences are immobilised to the support and are used to determine the sequence of non-immobilised polynucleotide. Thus there is a clear difference in the two methods, Singer immobilises undetermined sequences whilst claim 91 recites the immobilisation of predetermined sequences.

Applying polynucleotide probe in Singer would not give sequence, but would merely indicate that it contained a region complementary and capable of hybridising with another sequence within the fixed intact cell.

In view of the foregoing, favorable reconsideration and allowance is respectfully solicited.

Respectfully submitted,

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